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ICMSF methods studies. V. The influence of selective enrichment media and incubation temperatures on the detection of salmonellae in raw frozen meats[1,2,3]

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Sixty lots of frozen raw meat were analyzed for *Salmonella* using preenrichment in lactose broth followed by selective enrichment in four different selective broths, each incubated at 35C and 43C. Incubation at 43C resulted in detection of more *Salmonella*-containing samples. The highest recovery was observed with selenite cystine at 43C. However, the data indicated that two selective broths should be used for maximum recovery. Tetrathionate broth without brilliant green was inferior to the three other selective media used, both at 35C and 43C. Using selenite cystine, tetrathionate brilliant green, and selenite brilliant green sulfa broths, a number of medium/temperature pairs gave *Salmonella* recoveries approaching that obtained from all eight medium/temperature variables combined.

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A partir de 60 lots de viande crue congelée, on a recherché les *Salmonella* par une technique de préenrichissement en bouillon lactose suivi d'un enrichissement sélectif avec quatre bouillons sélectifs différents incubés respectivement à 35C et 43C. L'incubation à 43C permet de détecter un plus grand nombre d'échantillons contenant des *Salmonella*. Les meilleurs résultats sont obtenus avec le millieu sélénite-cystine à 43C. Les résultats suggèrent cependant d'utiliser deux bouillons sélectifs pour avoir un taux maximum d'isolement. Le bouillon tétrathionate sans vert brillant est inférieur aux trois autres milieux sélectifs utilisés et à 35C et à 43C. En employant les bouillons sélénite-cystine, tétrathionate-vert brillant, et sélénite-vert brillant-sulfa, un nombre de paires milieu/température a donné un taux d'isolement de *Salmonella* qui se rapproche de celui obtenu avec la totalité de huit différentes combinaisons milieu/température.
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Harvey and Thompson (6) were first to report increased recovery of salmonellae by incubation of selective enrichment broths at 43C instead of the more usually used 35–37C. Hobbs (7) found the reverse. Georgala and Boothroyd (5) studied 125 frozen meat samples using direct enrichment in selenite broth at 43C (the Colworth system) as was as by direct enrichment in both tetrathionate and selenite broths at 37C (the Food Hygiene Laboratory system) (9). Thirty positive samples were detected using selenite broth incubated at 43C, whereas 31 positive samples were detected using selenite and tetrathionate broths incubated at 37C. Edel and Kampelmacher (2) reported on comparative studies in eight European laboratories. Incubation of selective broths at 43C detected more *Salmonella*-containing samples than incubation at 37C, particularly in the analysis of samples having a high level of competing organisms. Erdman (3), reporting on an international collaborative assay on artificially inoculated raw meat, concluded that incubation of selective broths at 43C resulted in the detection of more positive samples than did incubation at 35C.

The present study reports on the analysis of 60 different lots of raw meat each of which was naturally contaminated with salmonellae. Four different selective enrichment broths were used with incubation of each at 35C and 43C. In a previous study (4) it was reported that preenrichment followed by selective enrichment resulted in increased recovery of salmonellae from raw meats. Accordingly all samples were preenriched before selective enrichment under the eight different conditions.

Materials and Methods

Samples

Ten different lots of six types of frozen meats were obtained from a pet-food manufacturer. The 60 samples in-

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cluded pork spleen, beef trimmings, pork lungs, whole chicken, beef liver, and pork kidney which were coarse-ground in normal production. Two-kilogram samples were randomly drawn from each lot and were held at −20C before analysis.

Preenrichment

The frozen samples were thawed overnight at 7C. For analysis, 357.5 g of each sample was weighed into a sterile blender jar and blended for 2 min at high speed with 400 ml of lactose broth containing 1% Tergitol (8). The homogenate was then added to 2817.5 ml of lactose broth containing Tergitol, and the resultant suspension was mixed with a glass stirring rod for 5 min. Three hundred and twenty-five milliliters of this mixed 1:10 dilution was aseptically transferred to a sterile 500-ml screw cap bottle to provide a 32.5-g sample; the remaining portion represented a 325-g sample. All preenrichment cultures were incubated 24 h at 35C.

Selective Enrichment

After incubation, the preenriched samples were thoroughly mixed, and 1 ml was introduced into duplicate tubes of each of the following media (Difco, Detroit, Michigan): selenite cystine, selenite brilliant green sulfa, tetrathionate, and tetrathionate brilliant green broths. Tetrathionate brilliant green broth was prepared as prescribed by Thatcher and Clark (10). One set of tubes was incubated at 35C, and the duplicate set was incubated at 43C, both for 24 h.

Selective and Differential Media

After incubation, each of the eight selective broths was streaked onto brilliant green (BG), *Salmonella-Shigella* (SS), and bismuth sulfite (BS) agars (Difco). The plates were incubated at 35C. The SS and BG plates were examined after 24 h, and the BS plates after 48 h.

Identification

Suspect colonies were identified by conventional biochemical and serological procedures (10). The serological identification included slide agglutinations using group-specific O antisera (Difco) and tube agglutinations using Spicer-Edwards H antisera (Difco).

Results and Discussion

A comparison of salmonellae recovery by the eight enrichment variables within each of six products is presented in Table 1. The data obtained from all eight variables within each product was subjected to analysis of variance using factorial Chi square (1). A typical analysis of variance summary using beef liver as an example is presented in Table 2. No significant differences were observed between the enrichment variables in pork spleen, ground beef, or pork kidney samples. In pork lung, ground chicken, and beef liver samples significant differences ($p = .001$, $p = .05$, $p = .001$ respectively) were observed which could be assigned to the temperature effect, that is, 43C incubation resulted in more positive samples being detected than 35C incubation. The pork lung samples yielded significantly fewer positive samples in tetrathionate broth and in tetrathionate broth containing brilliant green, both incubated at 35C as compared with the other enrichment variables. This would indicate a combined medium/temperature interaction which statistical analysis confirmed ($p = .001$).

TABLE 1

Comparative recovery of salmonellae in meat products using four selective media at 35C and 43C incubation

Product[a] category	Weight of aliquots, g	Total[b] number aliquots positive	Number of positive aliquots							
			Medium/Temperature[c]							
			1	2	3	4	5	6	7	8
Pork spleen	325	10	8	10	9	9	7	9	10	10
	32.5	9	7	7	6	8	7	6	7	9
Ground beef	325	10	9	9	8	9	9	9	8	10
	32.5	9	8	9	9	9	8	9	9	8
Pork lung	325	10	9	10	10	10	5	10	4	10
	32.5	10	8	9	7	10	6	10	8	10
Ground chicken	325	10	7	9	8	9	2	4	3	6
	32.5	5	4	5	4	4	3	3	2	4
Beef liver	325	8	3	6	5	5	3	4	1	3
	32.5	2	0	2	1	2	1	2	1	1
Pork kidney	325	4	4	4	4	4	4	4	3	4
	32.5	1	0	1	1	1	1	1	0	1
Total positives		88	67	81	72	80	56	71	56	76

[a]Ten samples in each product category were analyzed.
[b]Total number of positive aliquots based upon combined results from all eight conditions.
[c]Media/temperature: 1, selenite cystine/35C; 2, selenite cystine/43C; 3, selenite brilliant green sulfa/35C; 4, selenite brilliant green sulfa/43C; 5, tetrathionate/35C; 6, tetrathionate/43C; 7, tetrathionate brilliant green/35C; 8, tetrathionate brilliant green/43C.

TABLE 2

Summary of factorial Chi square analysis for *Salmonella* recovery in 10 beef liver samples using four selective media at 35C and 43C incubation

Comparisons	DF	X^2	Interpretation of significance
Main effects			
Aliquots (A)	1	13.333	Significant, $p = .001$, higher recovery in 325 g
Temperature (T)	1	13.333	Significant, $p = .001$, higher recovery at 43C
Medium (M)	3	3.773	Not significant
Interactions			
A×T	1	0.033	Not significant
T×M	3	3.667	"
A×M	3	3.299	"
A×T×M	3	0.367	"

Since *Salmonella* detection systems are usually designed for classes of foods and not particular foods within a class, it is necessary to consider the overall recovery data for the entire group of meat samples to obtain a proper perspective of the data. The combined results from all eight selective enrichment variables (Table 1) showed that 88 of 120 aliquots were positive for *Salmonella*. The highest recovery for any single condition was obtained with selenite cystine broth incubated at 43C. The next highest recovery rates were from 43C incubation of selenite brilliant

TABLE 3

Comparative recovery of salmonellae in meat products using paired media/temperature combinations

	Number of positive aliquots						
	Products						
Media/temperature combinations[a]	Pork spleen	Ground beef	Pork lung	Ground chicken	Beef liver	Pork kidney	Total
1–2	17	18	20	15	8	5	83
1–3	16	18	19	13	7	5	78
1–4	18	19	20	13	7	5	82
1–5	16	18	18	12	5	5	74
1–6	17	18	20	13	7	5	80
1–7	18	18	19	12	4	4	75
1–8	19	19	20	12	5	5	80
2–3	17	18	20	15	10	5	85
2–4	19	19	20	15	8	5	86
2–5	18	18	20	14	8	5	83
2–6	17	18	20	14	9	5	83
2–7	18	18	19	14	8	5	82
2–8	19	19	20	15	9	5	87
3–4	18	19	20	15	9	5	86
3–5	17	18	17	12	6	5	75
3–6	17	18	20	13	7	5	80
3–7	18	18	19	13	6	5	79
3–8	19	19	20	12	7	5	82
4–5	18	19	20	14	7	5	83
4–6	18	19	20	14	8	5	84
4–7	18	19	20	13	7	5	82
4–8	19	19	20	14	8	5	85
5–6	18	18	20	8	6	5	75
5–7	18	18	16	6	4	5	67
5–8	19	18	20	10	6	5	78
6–7	17	18	20	8	6	5	74
6–8	19	19	20	11	6	5	80
7–8	19	19	20	11	5	5	79

[a]Media/temperature combinations: 1, selenite cystine/35C; 2, selenite cystine/43C; 3, selenite brilliant green sulfa/35C; 4, selenite brilliant green sulfa/43C; 5, tetrathionate/35C; 6, tetrathionate/43C; 7, tetrathionate brilliant green/35C; 8, tetrathionate brilliant green/43C.

green sulfa (80 positives) and tetrathionate brilliant green (76 positives). In every instance, 43C incubation of a given medium effected a significantly higher recovery rate than 35C ($p = .001$).

It is well recognized that the use of more than one selective broth will increase the rate of *Salmonella* recovery. Accordingly, *Salmonella* recovery rates for all possible pairings of each temperature/medium variable were calculated for all 120 aliquots (Table 3). As a point of reference, selenite cystine broth incubated at 43C yielded 81 positives, the highest rate for a single condition. Combination of the results for selenite cystine broth at 43C with each of the other seven enrichment variables increased the recovery rate, the highest being the pairing with tetrathionate brilliant green at 43C (87 positives). This compares favorably with the combined recovery from all eight enrichment variables (88 positives).

Similarly enhanced recovery of salmonellae from all paired enrichment variables was observed. *Salmonella* isolation from enrichment variables varied from 56 (tetrathionate at 35C) to 81 (selenite cystine at 43C). For paired variables recoveries varied from 67 (tetrathionate and tetrathionate containing brilliant green at 35C) to 87 (selenite cystine and tetrathionate brilliant green at 43C). The mean rate of recovery from the 28 paired enrichment variables was 80.3% and from the eight single enrichment variables, 69.9%.

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